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Several studies examining transient stress and immunological functioning were performed. Measurement issues involving method of saliva collection for use in measuring secretory IgA was explored and differences between whole and parotid saliva were detected. Analyses of sIgA antibody to a novel antigen serially measured over several weeks were performed and relationships with psychological coping variables were tentatively observed. IL-2 and Natural Killer cell assays were developed and tested, and we found that NK assays could not be successfully run from cryopreserved cells. A study replicating and extending previous work with a transient stressor (examinations) was run. Although overall group results did not replicate some previous work, ipsative analyses revealed that subjects who became more anxious in response to the stress had higher levels of lymphocyte proliferation.

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Immune Function and Psychological Stress

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The purpose of this project was an understanding of the effects of a transient stressor on several immunological parameters. In particular, we explored two new methods for tracking response to stressors: secretory IgA antibody (sIgA ab) to a novel antigen and levels of the lymphokine, interleukin-2, synthesized in response to a T lymphocyte mitogen (Concanavalin A). In addition to these immune parameters, we examined other immune parameters which had previously been examined by others (i.e., peripheral lymphocyte proliferation to a T cell mitogen and natural killer cell activity). The investigation involved several studies aimed at ironing out methodological problems with the procedures and testing the resulting assays using a transient stressor paradigm.

Study 1: Examination of Whole versus Parotid Saliva with Transient Stressors

Recent studies in psychoneuroimmunology have shown that stressful situations influence a large number of immunological measures. One research focus has been on secretory IgA (s-IgA), considered the body's first line of defence against disease (Tomasi, 1971). In general, the saliva used for the assays in these studies is collected by asking subjects to drool into a collection container both prior to and after a particular stressor. It is then analyzed with radial immunodiffusion assays (RID) yielding a measure of the total concentration of IgA protein. Results from these studies have demonstrated a difference between pre-stress levels of s-IgA and post-stress levels.

There are several difficulties with this procedure which have been discussed by Stone et al. (1987). Firstly, due to the inverse relationship between saliva flow rate and s-IgA protein, changes in flow rate caused by stress could appear to indicate changes in immunocompetence when, in fact, none were present. Secondly, there are proteases in whole saliva which are synthesized by bateria present in the oral cavity and which are known to break down s-IgA protein (Brandzaeg, 1971) and which could result in a difference between s-IgA measured from whole vs. parotid saliva. Thirdly, the health implications of fluctuations in s-IgA protein concentration are unclear since considerable amounts of IgA protein may exist in saliva, but with little or no protective IgA antibody present.

This paper addresses these two issues. In a longitudinal study with 6 observations per subject, both whole and parotid saliva were collected in stressful and nonstressful conditions. In both kinds of collections, duration of collection was standardized so that flow rate could be determined and RID values adjusted according to flow rate. As parotid saliva is collected directly from the duct of one of the parotid glands, the saliva is not contaminated by bacterial protease which might alter the amount of IgA protein or antibody which is detectable in the sample. This allowed us to explore two questions: 1. What is the relationship between IgA in whole and IgA in parotid saliva? 2. Is there a differential impact of stress on whole vs. parotid saliva?

METHODS:

Subjects. Twenty one male subjects from SUNY at Stony Brook participated and completed the study.

Procedure. The stressors where administered during two visits and the order of presentation of the stress tasks was counterbalanced. Saliva samples were collected just before, immediately after, and three hours after both Stroop and the cold pressor task.

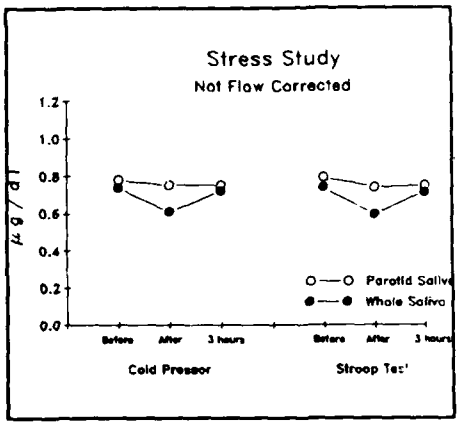
Stressors. Two stress tasks were used: Stroop's color word test (Stroop, 1935) and the cold pressor task. During Stroop subjects had first to name colors that where printed in black, then they had to identify various colors, and, lastly, they had to name the color a word was printed in when the word itself was a different color term. During the cold pressor task subjects immersed their right hand into 32°F ice cold water for two minutes.

Saliva. Unstimulated whole saliva was collected by having subjects drool into a cup for 3 minutes. Stimulated parotid saliva was collected by having subjects suck on a vitamin C tablet for one minute, at which time subjects swallowed the saliva and a Lashley cup was placed over their right Stenson's duct and saliva collected for three minutes. Whole saliva has generally been collected in other studies without stimulation of saliva flow, whereas it has been standard to collect parotid saliva with stimulation of saliva flow. The assay used to determine s-IgA protein concentration was radial immunodiffusion (RID) based on the method of Mancini, Carbonara, and Heremans (1965).

RESULTS:

1. What is the relationship between IgA in Whole and Parotid Saliva? Within-subject correlational analysis using a total of 6 measurements (right before, after and 3 hours after both the Stroop and the cold pressor task) of whole and parotid saliva indicated a weak relationship both when flow rate was ignored (mean Fisher Z $r = .08$, $p = ns$) and when flow was corrected for (mean Fisher Z $r = .13$, $p = ns$).

2. Is there a differential impact of stress on IgA in whole vs. parotid saliva? Repeated measure ANOVA was used to test whether or not the stressors decreased total s-IgA protein concentration when flow rate was not corrected for. There was one within factor based on time with three levels (saliva collected right before, right after, and three hours after the tasks). For whole saliva there was a significant main effect for time during both the Stroop task ($F(2, 38) = 15.98$, $p < .01$) and the cold pressor task ($F(2,38) = 10.30$, $p < .01$). The pattern is presented in the figure below. S-IgA concentration dropped right after both stressors as compared to S-IgA concentration immediately before and 3 hours after the stressors. On the other hand, in parotid saliva the main effect of time was not significant during either the Stroop task ($F(2,40) = 1.69$ $p = .20$) or the cold pressor task ($F(2,38) = 0.36$ $p = .69$). As can be seen in the figure, s-IgA concentration did not change during or after the stressors as compared to S-IgA concentration before and after the stressors.



DISCUSSION:

This study demonstrates that stress differentially affects s-IgA concentration in whole and parotid saliva. The lack of correspondence between whole and parotid saliva and the fact that s-IgA concentration decreased in whole saliva but not in parotid saliva after the stressors, indicates that factors other than decreased immunocompetence might be operating after the stressors. It is possible that flow rate affected s-IgA concentration in whole saliva as flow rate increased right after the stressors. As subjects had vitamin C to stimulate parotid saliva right before the stressors and the stressors took a short time, it is likely that the vitamin was still stimulating the salivary glands causing the increased flow. Given the inverse relationship between flow rate and s-IgA concentration, the increased flow might have caused the decrease in s-IgA.

Although these results are not definitive in recommending whole or parotid saliva for measuring s-IgA, future investigators should be aware that whole and parotid measures are not equivalent. Our own thoughts concerning s-IgA as a measure of immunocompetence (for the reasons stated in the introduction) are that it is not an ideal measure in measuring protection from invading organisms.

Study 2: Further Exploration of ONR-funded Data Sets: Coping and Immune Function

There has been much interest in whether and how stressful circumstances produce changes in physical health and, furthermore, what factors mediate the association. One of the most prominent psychosocial processes thought to mediate the impact of stress is coping. Some types of coping are thought to reduce the negative effects of stress whereas other coping strategies are thought to enhance or have no effect on the stress. Another potential mediator of the stress--illness relationship is physiological, the immune system, and it is thought by many to be the critical system for changing illness susceptibility. This study explores the relationships between reported stress from daily problems, coping with those problems, and secretory immunoglobulin A antibody (sIgA ab) in an exploratory effort to understand the mediational roles of coping and sIgA ab.

METHODS:

Subjects. Thirty dental students from the State University of New York at Stony Brook participated in this study. Men were studied so as to avoid any immunological changes associated with women's menstrual cycles. Subjects received $100 for their time. The average age of the students was 24.5 years.

Materials and Procedures. The psychological assessment was accomplished with a four-page booklet that was completed thrice weekly (Mondays, Wednesdays, and Fridays). For the purposes of this presentation, we discuss only the coping section of the booklet (mood and symptomatology sections were also present). Using a instrument developed for daily coping assessments (Stone & Neale, 1984), subjects briefly describe the most bothersome problem of the day, appraise the psychological characteristics of the problem using several questions (described below) and, finally, indicated whether or not they employed any of 9 methods of handling (coping) with the problem. The coping styles are described in the following table.

Coping Assessment Questions

1. DISTRACTION	Diverted your attention away from the problem by thinking about other things or engaging in some activity.
2. SITUATION REDEFINITION	Tried to see the problem in a different light that made it seem more bearable.
3. SOLUTIONS	Thought about solutions to the problem or gathered information about it.
4. DIRECT ACTION	Actually did something to try to solve the problem.
5. CATHARSIS	Expressed emotion in response to the problem to reduce tension, anxiety, or frustration.

6. ACCEPTANCE	Accepted that the problem had occurred, but that nothing could be done.
7. SEEKING SOCIAL SUPPORTS	Sought or found emotional support from loved ones, friends, or professionals.
8. RELAXATION	Did something with the explicit intention of relaxing.
9. RELIGION	Sought or found spiritual comfort and support.

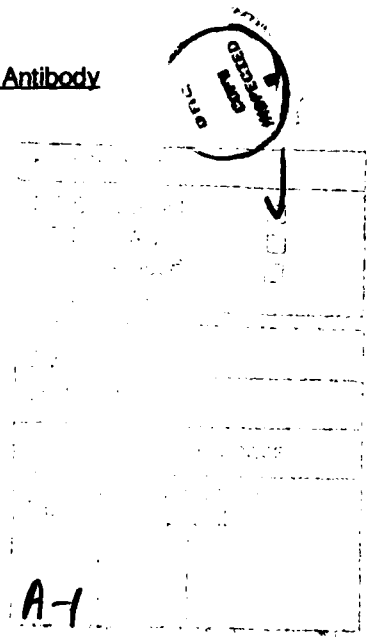
Throughout the course of the studies, on a daily basis subjects took a capsule containing purified rabbit albumin and sodium bicarbonate. We expected an immune reaction to this novel antigen that would be found in specific sIgA ab being produced in parotid saliva. At each of the 25 collection points, in addition to the questionnaire measures, we also collected a 3 minute sample of parotid saliva with a Curby cup. Secretory IgA protein concentration was assayed with radialimmunodiffusion (RID) and sIgA antibody activity was assayed with enzyme-linked immunosorbent assays (ELISA). Our dependent measure was the ratio of sIgA antibody divided by sIgA concentration in the saliva sample (see Stone et al., 1987).

RESULTS:

The first level of analyses was that of characterizing each individual by their average level of appraisal reporting, coping usage, and immunological response over the 25 reporting days. Correlations were then computed with each of the predictor variables (appraisals and coping measures) with the average level of sIgA ab reported (a logarithmic transformation of the raw antibody scores was computed to normalize the distribution). The nomothetic analyses indicate whether or not the average levels of response are related to one another, yet completely ignore the day to day fluctuations among the predictor and outcome variables. The table presents the means and correlations of predictors with ab.

Appraisal and Coping Means and rxy with Ab
Averaged Over Days

Measure	Mean (S.D.)	Correlation with Antibody
Uncontrolability of Problem	2.28 (.37)	-.23
Meaninglessness	2.11 (.47)	.11
Lack of control over resolution of problem	1.89 (.32)	-.09
Anticipation	2.77 (.39)	.12
Undesirability	4.48 (.68)	.10
Stressfulness	5.17 (1.28)	-.08
Distraction	.31 (.24)	.33 +
Situation Redefinition	.25 (.18)	.12
Solution	.34 (.22)	-.20
Direct Action	.58 (.20)	.02
Catharsis	.20 (.18)	.10
Acceptance	.20 (.17)	-.43 *
Social Supports	.18 (.20)	.19
Relaxation	.25 (.25)	.27
Religion	.04 (.12)	.19
Antibody (log)	-.17 (.15)	



+ $p < .10$ * $p < .05$

To address the within subject fluctuations, which is the real power of this design, we computed a regression analysis of the daily data on all individuals. To eliminate potential confounding of differential levels of response for individuals, response levels were controlled in the analyses. A further potential biasing factor was the growth in sIgA antibody over time, a phenomenon expected in the immunization protocol we employed and clearly shown in our previous analysis of this data (Stone, Cox, Valdimarsdottir, & Neale, 1987). To eliminate linear trends from the data due to growth in antibody, another control variable (referred to as Time) was added to the regressions prior to other predictor which represented experimental day.

The strategy of the analysis was to examine four sets of conceptually related predictor variables and to determine their ability to predict daily levels of sIgA antibody. Appraisals of the of most bothersome problem of the day was the first set to be examined, then the nine coping responses to the days problems. Interactions among selected appraisals and coping responses were examined next. A final set was evaluated the independent contribution of the sets of variables after the effects of daily negative and positive mood were statistically eliminated. This was done based on prior analysis of this data set where it was shown that daily moods predicted aggregated sIgA levels (Stone, Cox, Valdimarsdottir, & Neale, 1987).

Appraisals Of the original ten appraisal questions of the day's most bothersome problem, four were not considered in this analysis because they were not answered frequently enough (under 600 of 750 days) and their inclusion drastically reduced the number of analyzable days. When the problem occurred, whether or not it was chronic, if it had happened before, and how changing or stabilizing it was to the subjects's life styles were the four appraisals not included here. The next table presents the results of the regression of the remaining six appraisals predicting the log of daily sIgA antibody.

Problem Appraisals Predicting sIgA Antibody

Variable	Regression Weight
N = 542 R^2 = .45 (p < .0001)	
ID***	
Time	.041 ***
Uncontrollable occurrence of the problem	.011
Meaninglessness	.076 *
Lack of control over resolution of problem	-.011
Anticipation	-.010
Undesirability	-.003
Stressfulness	.020
N = 647 R^2 = .46 (p < .0001)	
ID***	
Time	.041 ***
Distraction	-.034
Situation Redefinition	.030
Solution	-.043
Direct Action	.021
Catharsis	-.048
Acceptance	-.030
Social Supports	-.022
Relaxation	-.014
Religion	-.015

Note. The effect of ID indicates the effect of differing levels of response by subjects and it has no regression weight.

* p < .05 ** p < .01 *** p < .001

The significant effects indicate that perceiving the problem as having little meaning is associated with higher levels of antibody. There was also a strong individual differences in level of antibody effect (ID), but this effect is controlled prior to evaluation of the subsequent variables. As predicted, the effect of time on antibody growth was extremely reliable.

Coping. The table above also presents the effects of the various coping responses predicting daily antibody levels. No main effects of coping on antibody were observed.

Conceptually, coping might have been expected to have main effects on antibody. However, another line of argument suggests that coping interacts with level of stressful appraisals (high levels demanding more or different types of coping) to produce buffering effects. Another set of analyses explored the potential interactions(buffering) of appraisals and coping. Rather than overly inflate an already high experiment wide alpha rate, only the significant appraisal dimension, meaningfulness, was explored for potential interactions with the coping responses. The regression was evaluated hierarchically, allowing the main effects to predict antibody level prior to the entry of the interaction (cross-product) effects of meaningfulness and the nine coping responses. Of the nine possible interactions, the interaction between meaningfuless and seeking solutions to the problem was significant (p .05). In order to understand the form of the interaction, a 2 (Solution coping) x 3 (Levels of Meaningfulness) figure was created with the means of sIgA antibody (log base 10) statistically adjusted for individual differences in antibody level and for time effects. The means indicate that when the coping response of seeking solutions to problems is used that there is only

a slight effect of the problems's meaningfulness on antibody response. However, when the coping mode is not used, meaningfulness is an important predictor of antibody: more antibody is present on days with slightly meaningful problems relative to days with either moderately or extremely meaningful problems. In other words, the use of solution as a coping mode is consistent with a positive buffering effect.

DISCUSSION:

These preliminary results with a small sample suggest optimism in future consideration of the potential mediators explored. The results were not especially strong because there was relatively low statistical power available for testing effects, but also because the variation in stressful situations in this homogeneous group of students was low. No major stressful situations occurred in the brief study period, so that finding any effects was quite positive.

Study 3: Development of Immunological Assays

During the project period, we worked on the development of the assay systems which were proposed in the original plan. The measurement of tritiated thymidine uptake by Con A stimulated peripheral blood T lymphocytes is a relatively standard assay procedure and was readily developed to our satisfaction. Error within triplicate analyses was controlled for by elimination of outlying results (15% of the mean).

We had also proposed to determine IL-2 concentrations in the supernatants of the Con A stimulated peripheral blood using an IL-2 sensitive line of cells (CTLL-2). The analysis of IL-2 in the manner was carried out on supernatant samples of Con A stimulated cells as originally proposed. Initially, we were unable to determine IL-2 levels in the samples due to inadequate sensitivity, but subsequently increasing the amount of culture supernatant which was assayed to a total of 25% of the total volume per well in the CTLL-2 wells allowed predictable analysis of IL-2. Due to the small number of samples which we finally achieved analyses of IL-2, the current data does not appear to be useful. However, we are presently including these analyses in comparisons with other data on IL-2.

We had originally proposed to examine natural killer cell activity in the dental patients which were included in the study. Following cryopreservation and thawing of the peripheral blood white blood cells, we discovered that predictable recovery of NK cell activity cannot be accomplished with present technology in spite of a publication to the contrary. In attempting to develop the NK assay from cryopreserved cells, we attempted a number of modifications to standard freezing techniques which are employed for WBCs. These included: 1. increasing FCS concentration to 40% 2. decreasing the rate of temperature drop to less than 1/2 degree centigrade 3. increasing the DMSO concentration 4. switching the FCS to synthetic media, and 5. faster thawing of the cells. All these procedures were to no avail and, after contacting two other laboratories which regularly work with NK cells, discovered that it was unable to be done in their hands as well. Although we repeated Locke's originally published procedure for performing the cryopreservation, we found it to be impossible to predictably retrieve NK activity. Since performing NK assays immediately upon collection of the blood samples was not only technically impossible and would also not allow comparisons longitudinally within subjects, we abandoned the idea.

Study 4: Effects of a Transient Stressor on Immune Function

The previous study provided us with new information about the assys for this study (in addition to other relationships that immune function has to other psychological variables). The goal of this study was to use these assays in a study of naturally occurring stressors and to observe any changes in immune function attributable to the stressor. We had originally proposed to use periodontal surgery as the transient stress by following individuals coming into the University's dental clinic for surgery. We attempted to implement this design, but found it untenable for several reasons. First, many of the patients we solicited for the study were simply too medically ill and immune system assays could have easily been compromised by their condition and treatments of the conditions. Second, flow of "healthy" periodontal patients who had agreed to participate in the study was simply too low to allow completion of the study. Finally, we underestimated the complexities involved with drawing bloods on these patients in consistent ways (e.g., surgeons were changing appointments, patients would not arrive promptly, patients were not feeling up to a post-surgery blood draw, etc.). Alternatively, we used another stressor that has previously been used in psychoimmunology studies: that of medical student examinations.

The hypothesis that the immune system can be affected by psychological factors such as stressful events has recently received considerable attention (see Ader, 1981; Jemmott et al., 1984; Rojers et al., 1979; and Schleifer et al., 1986 for reviews). One potential stress that has been studied extensively is that of students undergoing examinations. From a research design perspective, the examination model is convenient because many students (usually from the same class) may be recruited at one time, psychological and immunological measures can be collected in groups prior to and during the examination, and there is a low attrition rate given the high level of student interest in the studies.

Many studies have shown alterations in immunological functioning from baseline to examination period. The measures used have included lymphocyte responses to mitogen stimulation (Dorian et al., 1982; Glaser, et al., 1985), natural killer cell activity (Kiecolt-Glaser et al., 1984a), interferon production (Glaser, et al., 1986a), Epstein-Barr virus antibodies (Kiecolt-Glaser, Speicher, et al., 1984b), percentages of helper and suppressor T-cells (Glaser et al., 1986b), salivary immunoglobulin A concentration (McClelland et al., 1985), and salivary immunoglobulin A synthesis rate (Jemmott et al., 1983).

The most common finding is a change in immune function at the examination period relative to the baseline. Several studies have found a lower immune response at examination (for example, Kiecolt-Glaser et al., 1984; Glaser et al., 1985, Glaser et al., 1986b; Jemmott et al., 1983; McClelland et al., 1985; Workman et al., 1987). However, many studies contradict that finding. For instance, Glaser and colleagues (1986a) found increases in serum levels of immunoglobulins M, G, and A during examinations relative to baseline. Dorian and colleagues (1982) found that relative to a control group, students had higher numbers of B lymphocytes, total white cells, total lymphocytes, and AET rosettes, two weeks before an examination, and two weeks after the exam enhanced lymphocyte proliferation was observed. Finally, Baker and colleagues (1984) found an increase in the percentages of helper T-cells during an examination period. Studies have also appeared where no change in immune measures was observed at examination period. For instance, Kiecolt-Glaser's study (1984a) demonstrated no change in secretory IgA concentration in saliva, serum immunoglobulins G and M on C-reactive protein.

In this study we attempt to replicate the examination stress studies performed at Ohio State University (Glaser and Kiecolt-Glaser studies cited above) that used Lymphocyte Proliferation in response to mitogen stimulation as the dependent measure. Although the studies has been replicated within that setting, they should be replicated at other sites. Furthermore, the analyses of psychological measures and immunological measures has been limited to nomothethic (group) analyses in the previous studies. A goal of this study was, despite a relatively small N, to extend the analyses into the realm of ipsative (individual differences) analyses to further understand the nature of the stress--immune relationship.

METHODS:

Subjects. Volunteers from the first year medical school class at SUNY, Stony Brook participated on three occasions. We announced the study to the entire class (approximately 100 students) and 37 students agreed to participate. Based on judgments from the consent form signatures, 10 subjects were female, 24 were males, and the sex of 3 subjects could not be determined from their names.

Materials. The Positive Affect Negative Affect Scales (PANAS; Watson et al., 1988) is a mood adjective checklist that was used to provide information about positive and negative moods on the day it was administered. Reliability and validity of the PANAS are excellent (Watson et al., 1988) and one desirable feature of the scales is that they do not correlate highly with one another. The Symptom Checklist-90 (SCL-90; Derogatis, 1977) is a well-known and validated symptom checklist that taps both physical and psychological states. The anxiety and depression scales were administered so that direct comparisons could be made with prior studies of medical student exam stress.

Procedure. The first collection point was 11 days prior to a week of midterm examinations. At this time psychological questionnaires were administered, followed by the blood draw. The second collection point was immediately following an examination on the second day of exams, and the third collection point was 7 days later.

Mitogen Stimulation. 20 ml of peripheral blood was collected into Vacutainer heparinized tubes. The blood was immediately (within 5 hours) subjected to density gradient sedimentation on ficoll-hypaque (1.077 gms/ml; Sigma Chemical Co., St. Louis). The buffy coat was washed x3 with RPMI 1640 (supplemented with 25mM Hepes and 40% human AB^+ serum). The white blood cells were then frozen in a liquid nitrogen refrigerator (in RPMI 1640, 20% DMSO) at a rate not to exceed $1^{\circ}C$/min.

Upon thawing, the cells are washed x2 and adjusted to 2x106/ml in RPMI + 10% AB^+ 25mM Hepes, 2% Kanamycin, 2% Genramycin. 100ul of cells are added to each well of a microtiter dish together with 100 ul of 200 ul/ml Con A. 100ul of additional media is then added. The cultures are incubated for 5 days at 37° in 5% CO_2. After the fifth day, 10ul of ^3H-thymidine (s.a. 6.7 Ci/nmol; 100 uCi/ml) is added to the wells. The plates are incubated an addtional 6 hours, harvested on a Flow cell harvester and the ^3H counted in a LKB liquid scintillation counter, the results expressed as DPM.

RESULTS:

Subject Attrition. Several subjects' data were eliminated from the analysis. First, some subjects (N = 10) did not attend all three of the sessions. We retained subjects that completed at least Time 1 and Time 2 measures. Second, infection of the stored bloods yielded several samples (N = 7) where no cellular activity was observed and these samples were eliminated. This yielded a final sample of 20 subjects with acceptable Time 1 and Time 2 data.

Lymphocyte Proliferation Assays. Quintuplicate analyses of each sample were performed because there is considerable variability in lymphocyte stimulation replicates. The median statistic was chosen to reduce the influence of outlying replicate

values. Furthermore, replicate values were examined and if two values were relatively high and two were relatively low, that sample was eliminated from subsequent analyses as we had no way of knowing which were the correct values. This examination of replicates resulted in elimination of two samples. Finally, the data was expressed as discharges per minute (dpm) in stimulated culture minus the dpm in unstimulated culture.

Means of Psychological and Immune Variables at Time 1 and 2

Variable	Time 1	Time 2
Anxiety	.85 (.67)	1.57 (.74)***
Negative Affect	1.90 (.52)	2.37 (.73)**
Positive Affect	3.54 (.56)	3.02 (.53)***
Depression	.57 (.61)	.66 (.60)
Lymphocyte Proliferation	4.86 (.29)	4.82 (.30)

Note. *** p < .001 **p < .01 *p < .05 +p < .10

Psychological and Immunological Change Over Time. Each of the four psychological indicators of stress was examined with repeated measures ANOVA. The means and standard deviations are presented in the table above. Anxiety increased significantly ($F(1,19) = 15.2$, p.001) as did Negative Affect ($F(1,19) = 9.1$, p.01). A significant decrease was observed in Positive Affect ($F(1,19) = 20.6$, p.001). There was no significant change in Depression ($F(1,19) = .4$, p = n.s.).

Before analyses were computed on the Lymphocyte Proliferation data, the distribution of scores was examined since others (Schleifer et al., 1985) have transformed raw scores to normalize distributions. We found that the distribution was highly skewed and that a log to the base 10 transformation produced a much more normal distribution. The Lymphocyte Proliferation means at the two time periods did not differ significantly in the analysis ($F(1,19) = .1$, p = n.s.).

Simultaneous Relationships Between Psychological Measures and Lymphocyte Proliferation at Time 1 and Time 2. The next step in the analysis was to examine cross-sectional relationships among the psychological and immune variables separately at each assessment point. The next table presents the intercorrelations among the variables at each time.

The correlations among the psychological variables at Time 1 are as expected: the negative affect states directly correlate with one another while indirect relationships are observed between Positive Affect and the Negative Mood measures. None of the correlations between the psychological variables and Lymphocyte Proliferation are significant.

Correlations of Psychological and Immune Measures Time 1 and 2
Time 1 coefficients are above the diagonal, Time 2 below the diagonal

Time 1			Time 2		
	Anxiety	Negative A.	Positive A.	Depression	Lymphocyte Proliferation
Anxiety	-	.66**	-.27	.75**	-.02
Negative Affect	.75**	-	-.15	.53*	.32
Positive Affect	.17	.01	-	-.34	-.14
Depression	.34	.43+	-.36	-	-.05
Lymphocyte Proliferation	.52*	.46*	.43+	-.12	-

Note. *** p < .001 **p < .01 *p < .05 +p < .10

At Time 2, however, Positive Affect does not correlate negatively with Anxiety and Depression correlates much less strongly with Anxiety and Negative Affect. Most striking, however, are the strong positive associations between Anxiety, Negative Affect, Positive Affect, and lymphocyte proliferation. It should be pointed out that these are not measures of change in the psychological measures, but concurrent relationships.

Changes in Psychological Measures Associated with Changes in lymphocyte proliferation. Although there are several ways to explore changes over time in naturalistic studies, we have chosen a hierarchical regression approach (Cohen & Cohen, 1985) where lymphocyte proliferation at Time 2 serves as the dependent measure. To adjust the dependent measure for changes from baseline (Time 1), lymphocyte proliferation at Time 1 is entered into the equation first. To assess the impact of baseline levels of a psychological variable (Negative Affect, Positive Affect, etc.), its value at Time 1 is then entered. Finally, the psychological variable at Time 2 is entered and the percent of variance it explains is evaluated to test for change in the variable from baseline. Because the N is small, the regression equation was computed four times, once for each of the psychological variables.

The table below presents the Time 1 and Time 2 simple correlations, which formed the basis for the regression analyses. Surprisingly, the Time 1 to Time 2 correlations on the same measure (stability) are not high or significant for any of the psychological measures except Positive Affect ($r = .57$). lymphocyte proliferation at Time 1 had virtually no correspondence with itself at Time 2 ($r = -.06$). These results suggest that the stress experienced at Time 2 was not simply an additive phenomenon where those high in Anxiety, for instance, became much more anxious at examinations (which would have produced large, positive correlations). Only one Time 1 predictor of lymphocyte proliferation at time 2 was detected in these correlations, Positive Affect (.46). The following regression analyses controlled Time 1 influences on lymphocyte proliferation before evaluating the effects of change in psychological measures over time.

As expected from the simple correlations, Time 1 lymphocyte proliferation poorly predicted Time 2 lymphocyte proliferation (less than 1% of the variance was explained, p.10). For Anxiety, entering the variable at Time 1 contributed an additional 1.3% of variance explained (p = n.s.), however, Time 2 Anxiety added an additional 34.2% of explained variance, a highly significant increase (p.01). For Negative Affect, Time 1 added 8.1% (p = n.s.) and Time 2 added an additional 13.3% (p = n.s.). For Positive Affect, Time 1 added 20.9% (p.05), while Time 2 added only an additional 4.2% (p = n.s.). Finally, Time 1 Depression added 7.9% (p = n.s.) and Time 2 added virtually no additional variance to the prediction.

Overall, the regressions indicated two significant effects on adjusted Time 2 lymphocyte proliferation: Time 2 Anxiety and Time 1 Positive Affect. The regression coefficients in both cases are positive, indicating a direct relationship of Positive Affect and Anxiety with adjusted lymphocyte proliferation at Time 2.

Correlations Among Psychological and Immune Variables Across Time

			Time 2		
Time 1	Anxiety	Negative A.	Positive A.	Depression	Lymphocyte Proliferation
Anxiety	.33	.30	-.32	.47*	-.11
NegativeAffect	.48*	.41+	-.25	.63**	.25
PositiveAffect	.36	.17	.57**	-.08	.46*
Depression	.20	.09	-.19	.50	-.28
Lymphocyte Proliferation	-.11	-.34	-.04	.05	-.06

Note. *** $p < .001$ ** $p < .01$ * $p < .05$ + $p < .10$

NonParametric Analyses. Because the size of the sample is quite small, the data were also examined with nonparametric statistical techniques to confirm the parametric findings. Change scores on the psychological variables and lymphocyte proliferation were computed and subjects were classified according to a median split on all measures. Thus, the dichotomies represent relative increases and/or decreases over time in the variables depending upon the distribution of a variable and the

overall difference in average level at the two points in time. For example, because lymphocyte proliferation did not change over time, on average, the dichotomy largely represented subjects who increased their level of lymphocyte proliferation or decreased their level of lymphocyte proliferation at Time 2. Four 2 x 2 tables were created and cell frequencies were analyzed with Fisher's Exact tests.

For Anxiety, the test of the interaction within the table was highly significant ($p.01$) and indicated that subjects whose anxiety increased (relative to the other group) had higher levels of lymphocyte proliferation. The same pattern was observed for Negative Affect, although at a slightly lower level of significance (p.05). There was a trend for Depression (p.10), however, the direction indicated by the cell frequencies was opposite that of Anxiety and Negative Affect: more depression was associated with less lymphocyte proliferation. Finally, cell frequencies for Positive Affect were essentially random.

DISCUSSION:

Like medical students at Ohio State and other universities, students at Stony Brook University became distressed during an examination period. They became significantly more anxious and more negative and less positive in their moods. It is therefore reasonable to conclude that the examinations were stressful, although it must also be stated that neither this study nor the Ohio State studies employed a non-examination control group to assess possible extraneous factors that could affect psychological or immune functioning over time.

In comparing our results to the Ohio State results, the impact of the examinations can be discussed in terms of average differences relative to the variability. From time 1 to time 2, the Ohio State students had an average change of .40 Z-scores on Depression, while our group differed only .14 Z-scores. On the Anxiety measure of the SCL-90, however, relative change was comparable, with the Ohio State students changing .90 Z-scores and our subjects changing .92 Z-scores. Thus, our subjects became relatively less depressed than the Ohio State students, but evidenced the same degree of change in anxiety.

Unlike some previous examination stress studies (e.g., Dorian et al., 1982; Glaser et al., 1985; Workman et al., 1987), we observed no overall change in lymphocyte proliferation values. In fact, the change from Time 1 to Time 2 was so small (from 4.86 to 4.82 with a standard deviation of .30) that a possible explanation of our lack of findings, low statistical power, is unlikely. What, then, is different in this study and prior studies? One difference may be how values of lymphocyte proliferation were computed. It is unclear in prior studies how discrepant values in the triplicate or quadruplicate replications of each sample were handled. We took the median of the samples to reduce the impact of outlying scores and, in a few cases, eliminated samples where two scores were high and two scores were low. Prior studies have computed the mean of the replicates and do not specify how samples were eliminated. Although this is possible source of the difference in findings, it is an unlikely one, because the error in replicates would have to consistently fall in one direction to influence the average values.

Another possible explanation has to do with the how the lymphocyte stimulation assays were performed. The Ohio State group used three concentrations of mitogen (Concanavalin A) in the assay: 2.5, 5.0, 10.0 micrograms per well. From plots of that data it seems clear that smaller effects were observed between pre- and post-tests at higher levels of mitogen concentrations (a significant concentration by time interaction was reported). The concentration of mitogen that we used was 20 micrograms per well, twice as high as their highest concentration. We have selected this concentration based upon a series of experiments determined under our incubation and assay conditions which indicate that 20 ug/well consistently yielded results which were in the most sensitive region of the concentration curve for Con A concentration versus stimulation (i.e., gratest slope). Therefore, we believe that under our assay conditions, that 20 ug/well is the concentration of Con A which produces the most sensitive stimulation results. Other concentration used in other studies may, under their assay conditions, produce similar results. the differences observed relative to different concentrations of Con A could either be dur to differences in cell surface receptor density elicited by stress or may have indicated that one or more of the concentrations were not responsive due to with being too low or too high on the concentration versus stimulation curves in their laboratory. Nevertheless, the results of the Ohio State group are very interesting and, because of the reasons above, may not be able to be adequately compared to our results.

Although the group data above do not support previous work, the individual differences analyses provide a more powerful method of examining the effects of stress, especially in light of the one-group design that has been used by us and others. If the psychological variable is part of the causal chain affecting immune functioning, then change over time on the measure should be related to immune system change. Unfortunately, prior studies have usually not reported this kind of analysis. (It is possible to have group changes from Time 1 to Time 2, yet not detect the ipsative relationships just described.) Subjects who became relatively more anxious to the examinations had an increase in lymphocyte proliferation whereas those subjects who became less anxious or did not change in their level of anxiety had lower levels of lymphocyte proliferation. Positive Affect was also related to adjusted levels of lymphocyte proliferation, but in this case the prestress level was the important predictor. Subjects with higher level of Positive Affect prior to the stress had higher levels of lymphocyte proliferation during the stress

(simple correlation between Time Positive Affect and Time 2 lymphocyte proliferation was .46). A surprising result was the lack of any significant finding for the Depression scale. Although Time 1 Depression was a reasonable predictor of Time 2 lymphocyte proliferation (simple correlation of -.28), the regression results indicated no reliable effects of either Time 1 or 2 Depression.

In summary, although subjects in this study were clearly stressed by their examinations, no overall group effect of the stress on mitogen response was observed. Ipsative analysis, however, indicated that those students who experienced relatively more stress during the exams had higher levels of mitogen response relative to those who were relatively less stressed. Although this finding is in the minority of examination stress studies, at least compared with previously observed group effects, it must be noted that as far as we know other researchers have not employed the analytic approach used in this paper. Considering the importance of ipsative analyses for one group designs, with all of the threats to internal validity that they are known to have (Cook & Campbell, 1979), we urge other investigators to explore their examination data in this manner.

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